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Epidemiological survey of zoonotic helminths in feral cats in Gran Canaria island (Macaronesian archipelago-Spain)

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Abstract

The presence of zoonotic parasites in feral cats have been widely considered all over the world. In Gran Canaria (Macaronesian archipelago, Canary Islands, Spain) the number of feral cats has grown out of control in urban and rural areas. 48 of *Felis catus* captured in different Gran Canaria areas were studied. Animals were necropsied and several organs were systematically examined in order to collect and identify macroscopic parasites. In addition, coprological tests were done in 28 cats. There were no statistically significant differences in the prevalence rate among sex, age or capture area, showing an overall prevalence of helminths of 77.1%. The most common tapeworms were *Dipylidium caninum* (64.6%) and *Taenia taeniaeformis* (31.3%), followed by the nematodes *Toxocara cati* (20.8%), *Ancylostoma tubaeforme* (18.8%), *Aelurostrongylusabstrusus* (10.4%) and *Truchuris vulpis* (2.08%). We also find several eggs of *Alaria alata* in the small intestine of one cat (2.08%), being the first description of this trematode in cats in the Canary Islands. Aproximatelly, 40% of the studied cats harboured more than one parasite. High rates of zoonotic species found in these animals suggest the need of controling parasitic infections and preventive measures against them.

Keywords

Feral Cat, Macaronesian archipelago, Canary Islands, prevalence, zoonosis, helminths

Introduction

Although clinicians and researchers are aware about the role that feral and wildlife animals could have as potential reservoirs of many human diseases, the authorities and society are still not aware of this situation. Recent papers have highlighted that feral animals could potentially harbour many zoonotic parasites (Adams *et al*. 2008; Headley *et al.* 2012).

There are several surveys about the prevalence of parasites in feral animals from world-wide areas (Dubey *et al*. 2009; Stojanovic and Foley 2011). The increase of feral animals population close to the rural or urban areas may constitute the reservoir of many diseases that can be spread to human beings, as well as domestic animal species of our environment. (Beugnet *et al.* 2014).

Feral cats may become infected by several parasites, but helminths have shown the highest prevalence (Millán and Casanova 2009). Household cats may become infected by helminth cysts and eggs voided in the feces of feral cats and this may lead to human infection (Nichol *et al*. 1981a). Species such as *Toxocara* cati and *Toxascaris leonina* have an orofecal transmission cycle and produce infection by fecal contamination of food, water or the environment (gardens, sandpits and playgrounds) or direct contact (Overgaauw *et al.* 2009). *Ancylostoma* spp, has also been identified in cats (Millán and Casanova 2009; Coelho *et al.* 2011), representing another potential public health problem. *Aelurostrongylus abstrusus* has also been observed in feral cats from other countries such Spain and Portugal (Miró and Gómez 1999), producing respiratory problems. Recently, *Dirofilaria immitis* infection was diagnosed in household, as well as feral animals on the island of Gran Canaria (Morchón *et al.* 2004; Montoya-Alonso *et al.* 2011).

Gran Canaria is the second most densely populated island of Spain, as well as of Macaronesia, a collection of several archipelagos in the North Atlantic Ocean of the coast of Europe and Africa that belong to three countries: Portugal, Spain, and Cape Verde. Its geographical situation and the number of

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visitors (more than 2.2 million each year) are very important in terms of the potential for many of these parasites to be transmitted to humans, thus the zoonotic connection. In the countryside of Gran Canaria exists abundant population of feral cats that -as in other locations- do not receive any treatment against parasites, but they have access to food resources other than wild preys, such as farms or garbage bins; thus they usually being in good physical conditions and therefore these animals may survive with parasites (Millan and Casanova 2009). These factors and the difficulty in controlling the droppings of feral cats makes necessary to pay special attention to controlling parasites (ESCCAP 2010).

The study of the parasitological status of cats is important to establish the role of these animals as a potential hazard to human and animal health. Therefore, the aim of the present work was to determine the prevalence -by means of coproparasitological examinations and parasitological necropsy- and evaluate the parasites in feral cats in different areas of Gran Canaria.

Materials and Methods

In this study, 48 feral cats (*Felis catus)* captured in Gran Canaria (Canary Islands, Spain) during authorized predator control campaigns were studied. These animals were collected from different Gran Canaria locations following the scheme of climate zones described by Rodriguez-Ponce *et al.* (1995). Four different isoclimatic zones are found on Gran Canaria, ascending in altitude from the coast to the central peak of the island: Dry and Desert zone (DD), Dry and Steppe zone (DS), Temperate Mild zone (TM), and Temperate Cold zone (TC). All these areas were classified in two groups in relation to the climatic zone similarities; "coast area" (Dry zones: area number 1) and "central and top areas" (Temperate zones : area number 2) (Fig. 1).

Of the cats analyzed, 36 were adults (20 males, 16 females) and 12 juveniles (<12 months, 4 males, 8 females). Animals were anesthetized with a combination of ketamine (Imalgene ®, Merial, France) and xylazine (Rompun®, Bayer, Spain) and then humanely euthanized with intravenous injection of sodium pentobarbital (Dolethal®, Vetoquinol). This protocol was done following the recommendations approved by the Ethical Committee of the Veterinary Faculty of Las Palmas de Gran Canaria University (Protocol 52/2009).

Each animal was necropsied to examine different organs such as the esophagus, trachea, lungs, heart, stomach, small and large intestines. Parasites obtained from these organs were stored in 70% ethanol. For Cestodes, the specimens were stained with carmine, differentiated in acid-alcohol, dehy-

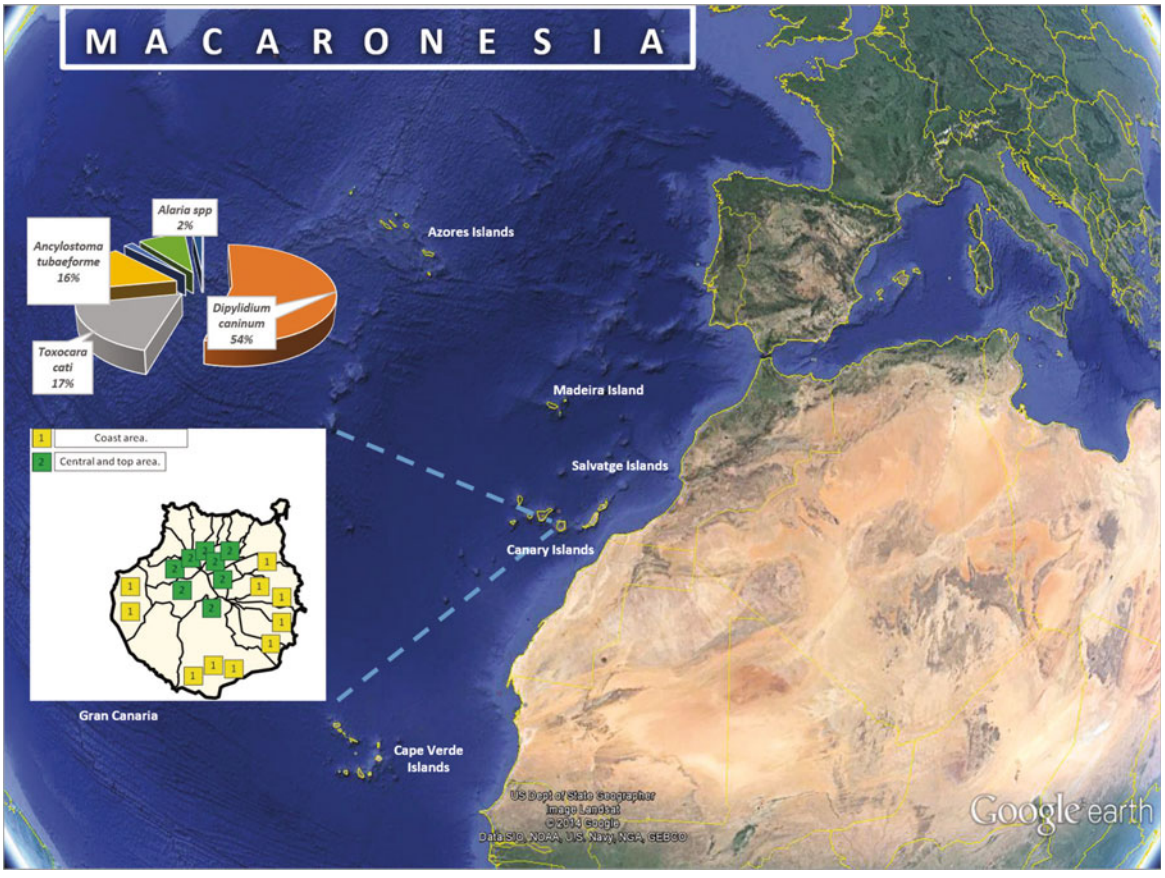

Fig. 1. Cartographical distribution of different regions where the feral cats were captured

Table I. Number and prevalence (%) of parasites in feral cats in the two capture areas of Gran Canaria lsland, Spain. Prevalence by groups and variables. Samples studied and overall prevalence according to epidemiological variables of each group

	Age	**Sex**	**Capture Area**	**Total**			
	>12 months (n = 36)	<12 months (n = 12)	Male (n = 24)	Female (n = 24)	Zone 1 (n = 18)	Zone 2 (n = 30)	
Cestodes	80.6% (n = 29)	58.3% (n = 7)	79.2% (n = 19)	70.8% (n = 17)	83.3% (n = 15)	70% (n = 21)	**75% (n = 36)**
Taenia taeniaeformis	33% (n = 12)	25% (n = 3)	25% (n = 6)	37.5% (n = 9)	22.2% (n = 4)	36.7% (n = 11)	31.3% (n = 15)
Dipylidium caninum	66.7% (n = 24)	58.3% (n = 7)	75% (n = 18)	54.2% (n = 13)	83.3% (n = 15)	53.3% (n = 16)	64.6% (n = 31)
Nematodes	16.7% (n = 6)	41.7% (n = 5)	33.3% (n = 8)	12.5% (n = 3)	16.7% (n = 3)	26.7% (n = 8)	**22.9% (n = 11)**
Toxocara cati	16.7% (n = 6)	33.3% (n = 4)	29.2% (n = 7)	12.5% (n = 3)	11.1% (n = 2)	26.7% (n = 8)	20.8% (n = 10)
Ancylostoma tubaeforme	13.9% (n = 5)	33.3% (n = 4)	29.2% (n = 7)	8.3% (n = 2)	11.1% (n = 2)	23.3% (n = 7)	18.8% (n = 9)
Trichuris vulpis	2.08% (n = 1)			2.08% (n = 1)	2.08% (n = 1)		2.08% (n = 1)
Aelurostrongylus abstrusus[1]	8.3% (n = 3)	16.7% (n = 2)	12.5% (n = 3)	8.3% (n = 2)	11.1% (n = 2)	10% (n = 3)	10.4% (n = 5)[1]
Trematodes	2.08% (n = 1)			2.08% (n = 1)	2.08% (n = 1)		**2.08% (n = 1)**
Alaria spp[2]	2.08% (n = 1)			2.08% (n = 1)	2.08% (n = 1)		2.08% (n = 1)[2]
Total Helminthes	**80.6% (n = 29)**	**66.7% (n = 8)**	**83.3% (n = 20)**	**70.8% (n = 17)**	**83.3% (n = 15)**	**73.3% (n = 22)**	**77.1%**

1: larvae stage in feces by coprological analysis
2: eggs in feces by coprological analysis

Table II. Number of parasites per animal in capture areas of Gran Canaria Island.

	Age		Sex		Capture Area		Total
	>12 months (n = 36)	<12 months (n = 12)	Male (n = 24)	Female (n = 24)	Zone 1 (n = 18)	Zone 2 (n = 30)	
Total parasites/cat							**48 (100%)**
0	19.4% (n = 7)	33.3% (n = 4)	16.7% (n = 4)	29.2% (n = 7)	16.7% (n = 3)	26.7% (n = 8)	11 (22.9%)
1	41.7% (n = 15)	8.3% (n = 1)	33.3% (n = 8)	33.3% (n = 8)	38.9% (n = 7)	30% (n = 9)	16 (33.3%)
2	19.4% (n = 7)	25% (n = 3)	16.7% (n = 4)	25% (n = 6)	27.8% (n = 5)	16.7% (n = 5)	10 (20.8%)
3	13.9% (n = 5)	25% (n = 3)	29.2% (n = 7)	4.2% (n = 1)	16.7% (n = 3)	16.7% (n = 5)	8 (16.7%)
4	5.6% (n = 2)	8.3% (n = 1)	4.2% (n = 1)	8.3% (n = 2)	0% (n = 0)	10% (n = 3)	3 (6.3%)
Zoonotic RiskParasite/cat*							
0	30.6% (n = 11)	33.3% (n = 4)	16.7% (n = 4)	45.8% (n = 11)	16.7% (n = 3)	40% (n = 12)	15 (31.3%)
1	52.8% (n = 19)	33.3% (n = 4)	54.2% (n = 13)	41.7% (n = 10)	72.2% (n = 13)	33.3% (n = 10)	23 (47.9%)
2	5.6% (n = 2)	8.3% (n = 1)	8.3% (n = 2)	4.2% (n = 1)	0% (n = 0)	10% (n = 3)	3 (6.3%)
3	11.1% (n = 4)	25% (n = 3)	20.8 (n = 5)	8.3 (n = 2)	11.1 (n = 2)	16.7% (n = 5)	7 (14.6%)

drated in serial concentrations of ethanol, cleared in xylene, mounted in DPX, and then identified morphologically to species according to existing descriptions and keys (Yamaguti 1958, 1959; Schmidt 1986). Nematode specimens were fixed in 70% (v/v) warm ethanol and stored in 70% ethanol containing 5% glycerol before being cleared by lactophenol and identified to species according to existing descriptions and keys (Skrjabin *et al.* 1991).

Faecal samples collected from the intestine were examined for parasitic infection. Ether-sedimentation and saturated sodium chloride flotation techniques were used to identify by light microscopy the parasitic dissemination elements.

Statistical analysis was done using SPSSâ (version 21.0). Chi-squared test (χ^2) was used to compare prevalence between age, sex or capture zone and parasite infection rates. Differences were considered significant at $p<0.05$. Confidence intervals (95 %) were calculated by means of exact Clopper–Pearson method.

Results

Most of the analyzed cats showed at least one species of helminth (77.1%). Seven helminth species (one trematode, two cestodes and four nematodes) were identified base on their morphology (Table I).

Cestodes were the most prevalent (75%) and 36 out of 48 cats were positive at least to one species of cestode, being *Dipylidium caninum* (64.6%) and *Taenia taeniformis* (31.3%) the only species observed. In the case of nematodes, the prevalence was 22.9% and there were four species detected: *Toxocara cati* (20.8%), *Ancylostoma tubaeforme* (18.8%), *Aelurostrongylus abstrusus* (10.4%) and *Trichuris vulpis* (2.08%) (Table I). Interestingly, *Dirofilaria immitis* was not detected in any of the animals of the present study.

High prevalence of zoonotic parasitic helminthes was found, and 68.7% of the cats were parasitized by at least one of the five potential zoonotic parasite found: 23 cats (47.9%) harbored a single zoonotic species, 3 cats (6.3%) harbored two zoonotic species and other 7 cats three zoonotic species. Only 22.9% were negative to any parasite (Table II).

The fecal samples done in 28 out of 48 animals showed the occurrence of single infections and co-infections by different species of parasites (58.3%). Five of these animals revealed the presence of L1 of *Aelurostrongylus* spp. However, in relation to trematodes, only one animal showed *Alaria spp*. eggs.

There were not significative differences between sex, weight and age, and captured areas. No relationship of captured areas with parasite prevalence was detected ($p = 0.5$).

Discussion

In touristic areas, close contact of cats with humans can increase transmission of zoonotic diseases and affect public health (Soriano *et al.* 2010). There are potential feline transmitted zoonotic parasites, such as *Dypilidium caninum*, *Toxocara* spp, *Ancylostoma tubaeforme and Dirofilaria inmmitis* that have been reported in different countries (Fenoy *et al.* 1997; Overgaauw 1997; Millán and Casanova 2009; Genchi *et al*. 2011). In the present study, the feral cats harbored five zoonotic parasites, *Ancylostoma tubaeforme*, *Toxocara cati*, *Dipylidium caninum*, *Taenia taeniformis* and *Trichuris vulpis*, showing a prevalence of 18.8%, 20.8%, 64.6%, 31.3% and 2.08% respectively. In Gran Canaria, as in many touristic areas, cats freely scavenge rubbish, gardens and street for food. In addition, tourists find that feeding these stray animals contribute to their welfare. This factor, couple with the public's poor understanding of disease transmission, and the hazards of pet faeces with respect to transmitting infectious diseases, underscores the importance of education in reducing the incidence of infections to avoid its transmission to other pets and humans.

Although numerous studies have described the worldwide prevalence of intestinal parasites in feral cats, only few published reports involved feline populations in the Canary Islands (Sánchez 2013). The overall prevalence of 77.1% of helminthes in this study was higher than that reported in other studies involving stray cats in Spain (Miró *et al.*. 2004; Torres *et al.* 2006), Portugal (23.1%) (Duarte *et al.* 2010), Germany (33.6%) (Becker *et al.* 2012) and the UK (34.8%) (Nichol *et al*. 1981b). However, our data are in accordance with those reported by Millán and Casanova (2009) in Majorca Island (Spain) –another popular Spanish tourist destination in Europe- where all the 58 feral cats were parasitized by at least one specie of helminth. This high prevalence could suggest a lack of parasite control and easier access to intermediate hosts as have been reported in other studies (Calvete *et al.* 1998; Millán and Casanova 2009).

In relation to the detected species in this study, *Dipylidium caninum* (64.6%), followed by *Taenia taeniaeformis* (31.3%) showed the highest prevalence. The abundance of cestodes has been reported in the small intestine of dogs, cats, mice and wild carnivores since 1959 by Jimenez and continues to be valid nowadays (Calvete *et al.* 1998; Millán and Casanova 2007, 2009).

The ascarid, *Toxocara cati* (20.8%) and the hookworm, *Ancylostoma tubaeforme* (18.8%) were the most commonly detected nematode. Necropsy surveys have shown that these worms represent the most common intestinal helminth parasites of cats worldwide (Barutzki and Schaper 2003; Ferreira *et al.* 2011), both with zoonotic potential. The prevalence of *Toxocara* infections in our study was similar to that found in stray cats and animal shelters (Calvete *et al.* 1998; Robben *et al.* 2004; Gracenea *et al.* 2009; Millán and Casanova 2009). However, lower prevalence has been observed in faecal analysis in households cats (Overgaauw *et al.* 2009), which is probably related with a lower exposure rate and the preventive deworming treatments. In the case of *Ancylostoma tubaeforme*, the prevalence found in this study was 18.8%. Other

studies on feral cats elsewhere have found similar results (Coman *et al.* 1981; Shaw *et al.* 1983; Vanparijs *et al.* 1991). In contrast, higher prevalence (91%) was observed in the gastrointestinal tract of cats analysed in Balearic Island, Spain (Millán and Casanova 2009). These two helminth species (*Toxocara* and *Ancylostoma*) may produce several larva migrans syndromes, including severe ocular or neurological damage in humans in case of Toxocara (Lee *et al.* 2010). Data obtained in this survey suggest that cats may constitute a potential source of infection to domestic animals and humans and can contribute significantly to the environmental burdens of these parasites in rural and urban areas. This fact has been recognized by several authors as being a significant feature in the spread of zoonotic and animal parasites (Széll *et al*. 2013).

Alaria spp. was found in this survey parasitizing the small intestine in one of the feral cats. This could be the first report of the presence of *Alaria* spp. in the Macaronesian area. However, we only detected the presence of *Alaria* spp. eggs in faeces, but not any parasite adults were observed in the intestine. This finding could suggest a pseudoparasitism originated by predation of some prey. Infestations by trematodes of genus *Alaria* are common in wild carnivores in many european countries (Criado-Fornelio *et al.* 2000; Wolfe *et al.* 2001; Segovia *et al.* 2003; Moks *et al.* 2006; Saeed *et al.* 2006). Infection are generally non-pathogenic for cats and dogs (Zajac and Conboy 2006). Although there are not been reported human cases of alariosis in Europe (Rentería-Solís *et al.* 2013), it is evident that a zoonotic risk can be presumed and a potential danger of this parasite has to be taken into consideration (Mohl *et al*. 2009; Portier *et al.* 2011). Therefore, the German Federal Institute for Risk Assessment (BfR), in its opinion No.027/2007, 1 July 2007 (Anonymous 2007), emphasized the pathogenic potential of this parasite.

Aelurostrongylus abstrusus was found in the faeces of five of the analysed animals (10.4%). This is the first description of these lungworms in the Canary Islands (Spain). Bronchopulmonary infestations by *A. abstrusus* have been well documented in cats throughout most European countries with similar prevalence rates that ranged from 1% to 24.4%, according to sampled populations and detection procedures (e.g., copromicroscopy or molecular detection) (Grandi *et al.* 2005; Taubert *et al.* 2009; Jefferies *et al*. 2010; Traversa *et al.* 2010). In the present study, lizards and birds were found in the digestive tract of our animals, suggesting that they could act as paratenic hosts for many metastrongylid species when they are predated upon by cats, as has been reported by Jefferies *et al.* (2010). Recent reports have indicated that infection in cats may be much more common than previously thought in endemic areas of Europe (Payo-Puente *et al.* 2008; Traversa *et al.* 2008, 2010). Moreover, emphasis should be placed on the diversity and potential for co-infections of cat metastrongylid species in Europe (Jefferies *et al.* 2010).

Interestingly, in spite of the high prevalence of *Dirofilaria immitis* in dogs from Gran Canaria, this parasite was not detected in any of the heart samples examined. Nevertheless, reports done by Montoya-Alonso *et al.* (2011) in cats from Gran Canaria island showed high seroprevalence (33%) using a commercial ELISA test kit. This high percentage could be due to tests used for the detection of circulating antibodies may overestimate the number of infected cats, with a high number of false positives (Berdoulay *et al.* 2004).

In conclusion, the present study demonstrated that the prevalence of parasites in feral cats in Gran Canaria island was high. These animals are reservoir of helminthes that may affect their health status and increase the zoonotic risk. We did not found differences between age, sex and captured areas parameters, suggesting a widespread distribution of helminth infective forms. Rates of intestinal and lung parasites and of zoonotic species found in feral cats in this study suggest the need of controlling these parasitic infections through regular diagnostic testing, effective therapeutic protocols and preventive measures.

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Conflict of interest
The authors declare no conflict of interest

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